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SIZE INHERITANCE AND GROWTH IN A MOUSE SPECIES CROSS (*MUS MUSCULUS* × *MUS* *BACTRIANUS*)

II. BIRTH WEIGHTS¹

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Birth weights, like litter size, are determined by the interaction of two sets of factors: one, intrinsic, or genetic, and the other, extrinsic, or environmental.

Within the limits of normal variation, induced by environmental factors, the weight at birth of any mammal is characteristic of that particular form. The newly born offspring of the Perissodactyla and the Artiodactyla are large and well developed, while, on the other hand, bears, and especially the Marsupialia, have extremely small, almost fetal, young. Within a single order considerable variation in size and precocity may occur. Among the Lagomorpha, the hares (*Lepus*) give birth to young fully furred and comparatively large, while the rabbits (e.g., *Oryctolagus* and *Sylvilagus*) produce blind and naked offspring. In the order Rodentia even greater differences occur. Guinea-pigs (*Cavia*) are large and so well developed at birth as to be nearly capable of independent existence. Mice (*Mus*), on the contrary, typifying the more usual situation in this order, are comparatively small at birth and are blind, naked, and helpless. Kopeć ('24), in comparing different races of rabbits, states that birth weight is in direct proportion to the average weight of the adult females of the breeds examined. In mice a similar

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condition prevails. Thus, while the size and degree of development at birth are not characteristics of the larger taxonomic divisions, such as classes and orders, they are, in so far as the genetic basis is concerned, specifically and racially constant. Kopeć ('26) considers birth weights of rabbits in general to be expressions of heredity and consequently of great morphogenetic value.

Among the environmental factors which have been held most markedly to affect the birth weight of a mammal are: size of the litter of which the individual is a member, weight of the mother, age of the mother, physical condition of the mother, length of gestation period, position of litter in the litter series, and season of the year.

There are a considerable number of data in the literature concerning the effects of litter size on birth weight of laboratory mammals. Kopeć ('24) remarked on the existence of an essential negative correlation between the average weight of newborn young and litter size in rabbits. King ('15), Hanson and Sholes ('24), and Feldman ('26) found that the same situation prevails in rats. Wright ('22) believed the size of litter to be an important factor in determining the variations in birth weight of guinea-pigs. Several workers, including Gates ('25), Parkes ('26), and Bluhm ('29), have observed that the birth weights of mice tend to vary inversely with the litter size.

Among rodents the weight of the dam also has some effect on the birth weight of her offspring. Bluhm ('29) gave the correlation between average birth weight and mothers' weight in mice as $+0.41 \pm .015$. She considered maternal weight even more important than litter size in determining birth weight. King ('15) stated that in rats the body weight of the dam influences the newborn weight of her young chiefly because it depends on age and physical condition. She observed that the birth weight varied directly with the age of the mother. Bluhm likewise found maternal age and birth weight correlated in mice, but held the influence of age to be limited. Wright ('22) stated that in guinea-pigs the age of the dam is not an important factor.

Kopeć ('26) studied the birth weight of rabbits and listed the physical condition of the pregnant female as one of the factors influencing the growth of the fetus and consequently the birth weight. King ('15) observed that female rats in good condition usually bore heavier offspring.

The length of the gestation period is another factor affecting birth weights. Kopeć ('24) obtained evidence indicating that the weight of rabbits at birth is in direct proportion to the length of the gestation period. King ('15), working with rats, arrived at the same conclusion. Wright ('22) found large litters associated with short gestation periods in guinea-pigs, so the lower birth weights of the members of large litters may be partly due to the briefer fetal life.

King ('15) observed that the birth weight of rats varied directly with the ascending scale of litter series, remarking, however, that the litter series is always an age series. Bluhm ('29) considers the position of the litter in the litter series to have comparatively little effect on the birth weight of mice.

Hanson and Sholes ('24) remarked some slight differences in the birth weights of albino rats born at different seasons of the year, but none was large enough in respect to its probable error to be considered significant. Bluhm ('29) reported that mice born in winter are 4 per cent heavier than those born in summer (1.23 and 1.18 grams, respectively), and in view of the large numbers involved, this finding is possibly statistically significant.

Summarizing: The birth weight of laboratory mammals depends: first, upon heredity (genetic factors) and, secondly, upon environmental influences. Of the latter, litter size and nutrition of the mother—of which maternal weight is an expression—appear of most importance. Where sampling is non-selective, however, either all litters from a given mating being included or else chosen at random in close agreement with the normal distribution of litter sizes for the stock under consideration, the comparison of average birth weights of the offspring from series of litters must approximate a comparison of the genetic constants for those series. The influence

of litter size, then, must be comparatively insignificant. Such has been the case in the present problem. Likewise, in our series nutritional conditions of the females have been maintained as closely as possible at a constant high level and there have been no marked differences in the age groups of the females employed. Thus, it seems probable that differences shown in our mean birth weights are genetic in nature and are innate rather than environmental.

Among rodents, males seem to be slightly heavier than females at birth. King ('15) and Feldman ('26) remarked this condition in rats. Wright ('22) obtained evidence for a like tendency in guinea-pigs. Our data, showing that male mice have a consistently greater birth weight than do females, are at variance with the conclusions of Gates ('25), who found no sexual difference in this respect.

The forms used in this study were the Little inbred strain of dilute brown, non-agouti *Mus musculus* and a race of *Mus bactrianus*, the original animals of which were captured near Peiping, China, and brought to the United States by Dr. Sheo Nan Cheer in 1926. Specimens of this stock have been identified by Dr. G. M. Allen as intermediate between the Gobi Desert race, *Mus bactrianus gansuensis*, and the race inhabiting western China, *Mus bactrianus tantillus*, although on the whole nearer the former. Our strains of *musculus* and *bactrianus* differ in many respects, among which are size, color, relative tail length, and litter size. *Musculus* adults average about 30 grams in weight, while *bactrianus* animals attain approximately half that figure. The average litter size of the former is $5.4 \pm .04$ (1400 litters); that of the latter is $4.6 \pm .11$ (111 litters). These species mate readily, and the offspring of both sexes and of reciprocal matings are fertile.

The method of procedure has been kept constant throughout the experiment. The isolated pregnant females were examined and all litters recorded at approximately the same time every morning. The total number of young in the litter, the number of stillbirths, and the sex of each individual were recorded in a ledger. In order to identify the members of a

litter before they were old enough to receive their permanent earmarks, an adaptation of the method of branding employed by Gates ('25) was adopted. One, two, and three of each litter were touched with the tip of a red-hot wire on the left shoulder, left side, and left hip, respectively; four, five, and six, on the right shoulder, right side, and right hip; seven, eight, and nine, between the shoulders, the middle of the back, and the base of the tail; ten, eleven, and twelve received double marks on the left shoulder, left side, and left hip. In every instance this brand healed rapidly, leaving a clean scar which remained throughout the life of the animal. Each mouse was weighed individually to the nearest 0.01 gram immediately after branding. At the age of ten days the identification of the sex was confirmed and, in the case of the back-cross generation, the coat color was recorded. Since the coat color of a mouse cannot be determined with certainty much before the age of ten days and since the sex can then be recognized unmistakably, only those individuals living to that age were included in the study of birth weights, in order to make all matings comparable.

That such a procedure has not acted selectively is indicated by an earlier incomplete tabulation of all individuals in the back-cross generation produced by mating F_1 females to musculus males. The mean birth weights of males and females were $1.32 \pm .011$ and $1.29 \pm .012$, respectively, while the means obtained from the complete tabulation of all animals from such matings living to the age of ten days are $1.35 \pm .008$ and $1.29 \pm .009$.

The term 'birth weight' is really something of a misnomer, since, as here employed, it actually means the weight of living mice at an age of less than twenty-four hours. The majority of litters of young mice are apparently born early in the morning, so most of the animals were but a few hours old at the first weighing. Because of the ingestion of milk, this weight is often somewhat in excess of the actual birth weight. The relative error involved, however, is not selective and, since it affects all our matings alike, may be disregarded.

Table 1 gives the mean birth weights of the parental stocks and the F_1 generations. The average weight of the males from musculus $\times$ musculus matings, regardless of litter size, is $1.40 \pm .012$ grams, and of the females, $1.36 \pm .011$. The difference ($0.04 \pm .16$) is slight and taken by itself is scarcely significant. The birth weight of pure bactrianus males, irrespective of litter size, is $1.15 \pm .010$; of the females, $1.11 \pm .010$. This sexual difference is likewise slight ($0.04 \pm .014$). The means for the F_1 generation (musculus $\text{♀} \times$ bactrianus ♂) are as follows: males, $1.36 \pm .011$; females, $1.32 \pm .014$. The difference is $0.04 \pm .018$ gram. In the reciprocal cross (bactrianus $\text{♀} \times$ musculus ♂) the males weigh $1.38 \pm .014$ grams and the females, $1.32 \pm .014$ grams—a difference of

TABLE 1
Mean birth weights

MATING	MALES		FEMALES	
	Number of individuals	Mean weight (in grams)	Number of individuals	Mean weight (in grams)
Musculus $\text{♀} \times$ musculus ♂	67	$1.40 \pm .012$	64	$1.36 \pm .011$
Bactrianus $\text{♀} \times$ bactrianus ♂	86	$1.15 \pm .010$	97	$1.11 \pm .010$
Musculus $\text{♀} \times$ bactrianus ♂	85	$1.36 \pm .011$	86	$1.32 \pm .014$
Bactrianus $\text{♀} \times$ musculus ♂	24	$1.38 \pm .014$	27	$1.32 \pm .014$

$0.06 \pm .020$ gram. The F_1 young at birth are nearly as heavy as the offspring of the heavier musculus stock. Kopeć ('24 a) found the newborn weight of F_1 rabbits to be intermediate between the newborn weights of the parent breeds. Thus, the large size of our F_1 animals may be explicable by heterosis. Wright ('22 a), after an extensive experiment in crossing inbred races of guinea-pigs, reached the conclusion that the breeding of the dam counted for about three-fourths in determining the birth weight of the offspring, while the genetic complex of the fetus contributed the other one-fourth. Our hybrid animals from mating of musculus females and bactrianus males would appear to confirm this conclusion. The fact, however, that the offspring of the reciprocal cross weigh fully as much makes it seem that the genetic composition of the growing fetus plays a greater part in determining the birth

weight of mice than of guinea-pigs, since such a situation can scarcely be explained by the slightly greater weight due to the smaller litters of the bactrianus females.

As a check on the permissibility of including young from all litters regardless of size in our summaries, means were computed for the birth weights of young from modal litters only in several of our matings. The most frequent litter sizes in the pure musculus stock were five and six; the most numerous in pure bactrianus also five and six, while the modal litter size in the back-cross generation (F_1 females $\times$ musculus males) was five. The mean birth weights for these modal litters, together with means for litters of all sizes, are listed below.

TABLE 2

MATING	NUMBER OF INDIVIDUALS	MEAN WEIGHT MALES	NUMBER OF INDIVIDUALS	MEAN WEIGHT FEMALES
Musculus ♀ $\times$ musculus ♂				
All litters	67	$1.40 \pm .012$	64	$1.36 \pm .011$
Modal litters only	25	$1.44 \pm .020$	26	$1.35 \pm .016$
Bactrianus ♀ $\times$ bactrianus ♂				
All litters	86	$1.15 \pm .010$	97	$1.11 \pm .010$
Modal litters only	48	$1.12 \pm .013$	63	$1.10 \pm .011$
<u>Musculus</u> Bactrianus ♀ $\times$ musculus ♂				
All litters	247	$1.35 \pm .008$	203	$1.29 \pm .009$
Modal litters only	33	$1.33 \pm .019$	36	$1.31 \pm .016$

The remarkable similarity of birth weights of mice from litters regardless of size and from modal litters only is striking. Apparently the inclusion of all litters gives as true a picture of the genetic basis of birth weight as the use of only the modal litters, so throughout the report the birth weights are computed from data on all litters irrespective of size.

Since the back-cross generation consists of matings of two distinct types, those in which the females were musculus and the males F_1 animals, and those in which the females were F_1 's and the males musculus, each will be considered separately in order to disclose the differential effects—if any—of maternal inheritance. All F_1 animals employed in produc-

ing the back-cross generation were from musculus females mated to bactrianus males. The mean birth weights for the two divisions are shown in tables 3 and 4. Male and female offspring from musculus dams weighed $1.40 \pm .012$ and $1.34 \pm .012$ grams, respectively; the mean weights for the young of hybrid mothers are $1.35 \pm .008$ and $1.29 \pm .009$ grams. In both instances males are significantly heavier than females.

TABLE 3
Mean birth weights, *musculus* ♀ × *bactrianus* ♂

CLASS	MALES		FEMALES	
	Number of individuals	Mean weight (in grams)	Number of individuals	Mean weight (in grams)
All classes	137	$1.40 \pm .012$	125	$1.34 \pm .012$
One-factor difference				
Agouti (A ^w)	71	$1.42 \pm .015$	59	$1.35 \pm .018$
Non-agouti (a)	66	$1.39 \pm .020$	66	$1.34 \pm .018$
Black (B)	63	$1.39 \pm .017$	62	$1.33 \pm .017$
Brown (b)	74	$1.42 \pm .017$	63	$1.34 \pm .018$
Intense (D)	64	$1.40 \pm .018$	51	$1.38 \pm .019$
Dilute (d)	73	$1.41 \pm .017$	74	$1.32 \pm .016$
Two-factor difference				
Black agouti (BA ^w)	33	$1.45 \pm .018$	30	$1.36 \pm .025$
Brown non-agouti (ba)	35	$1.43 \pm .026$	34	$1.32 \pm .024$
Black intense (DB)	34	$1.41 \pm .026$	35	$1.39 \pm .024$
Brown dilute (db)	43	$1.43 \pm .024$	46	$1.34 \pm .021$
Intense agouti (DA ^w)	35	$1.42 \pm .020$	25	$1.40 \pm .022$
Dilute non-agouti (da)	37	$1.40 \pm .026$	39	$1.32 \pm .022$
Three-factor difference				
Intense black agouti (DBA ^w)	17	$1.48 \pm .024$	17	$1.41 \pm .028$
Dilute brown non-agouti (dba)	23	$1.44 \pm .034$	25	$1.34 \pm .030$

When the mother is musculus, the male offspring average $0.05 \pm .014$ gram and the female offspring $0.05 \pm .015$ gram heavier than when she is an F₁ hybrid. When it is recalled that the musculus mothers of the back-cross generation averaged 5.3 young per litter and the hybrid females averaged 6.4 young, this difference is easily understandable, since litter size is one of the most important environmental factors affect-

ing birth weight. These results, then, do not necessarily conflict with the findings for the F_1 generation and are not antipathetic to the possibility that in mice the genetic constitution of the growing fetus plays a very important part in determining the weight at birth.

TABLE 4
Mean birth weights $\frac{musculus}{bactrianus} \text{♀} \times musculus \text{♂}$

CLASS	MALES		FEMALES	
	Number of individuals	Mean weight (in grams)	Number of individuals	Mean weight (in grams)
All classes:				
All litters	247	$1.35 \pm .008$	203	$1.29 \pm .009$
Modal litters only	33	$1.33 \pm .019$	36	$1.31 \pm .016$
One-factor difference				
Agouti, (A^w)	117	$1.35 \pm .011$	113	$1.30 \pm .012$
Non-agouti (a)	130	$1.34 \pm .011$	90	$1.29 \pm .013$
Black (B)	109	$1.36 \pm .012$	98	$1.28 \pm .013$
Brown (b)	138	$1.34 \pm .010$	105	$1.31 \pm .012$
Intense (D)	126	$1.34 \pm .011$	107	$1.31 \pm .011$
Dilute (d)	121	$1.35 \pm .011$	96	$1.27 \pm .013$
Two-factor difference				
Black agouti (BA^w)	58	$1.35 \pm .016$	58	$1.30 \pm .018$
Brown non-agouti (ba)	79	$1.32 \pm .014$	51	$1.32 \pm .019$
Black intense (DB)	48	$1.35 \pm .018$	52	$1.29 \pm .018$
Brown dilute (db)	60	$1.35 \pm .015$	50	$1.28 \pm .019$
Intense agouti (DA^w)	59	$1.35 \pm .016$	54	$1.32 \pm .018$
Dilute non-agouti (da)	63	$1.36 \pm .015$	38	$1.27 \pm .023$
Three-factor difference				
Intense black agouti (DBA^w)	21	$1.35 \pm .025$	30	$1.31 \pm .027$
Dilute brown non-agouti (dba)	39	$1.34 \pm .018$	20	$1.30 \pm .034$

If the birth weight of a mouse is an expression of quantitative genetic factors, as it seems unquestionably to be—whether or not it is of such morphogenetic value as Kopeć ('26) found to be the case in rabbits—then the back-cross generation offers an opportunity for the testing of linkage between qualitative and quantitative characters. The bactrianus parent possesses the dominant genes, D, B, and A^w (mouse club symbols, Cuénot, '28), while the dilute brown musculus parent exhibits

the three recessive allelomorphs, d, b, and a. Thus, the back-cross generation consists of eight phenotypes in equal numbers; intense black agoutis (DBA^w), dilute black agoutis (dBA^w), intense brown agoutis (DbA^w), dilute brown agoutis (dbA^w), intense black non-agoutis (DBa), dilute black non-agoutis (dBa), intense brown non-agoutis (DbA), and dilute brown non-agoutis (dba). If genes for high birth-weight values are linked with these recessive genes affecting coat color, the extracted recessive animals (in regard to coat color) should be heavier at birth than their dominant allelomorphs. This can be tested by tabulating birth weights according to the coat color the animals later developed. Table 3 presents a summary of such tabulations for one type of back-cross mating.

If one or more major size factors be linked with one of the recessive genes, the condition may be detected by contrasting the mean birth weight of the mice possessing the recessive gene to the mean birth weight of those not possessing it. These values for all mice having the gene for agouti (A^w) are $1.42 \pm .015$ and $1.35 \pm .018$ grams for males and females, respectively, while the non-agouti individuals weighed $1.39 \pm .20$ and $1.34 \pm .018$ grams. Obviously, there is no significant relationship—in fact, the mice possessing the dominant gene are slightly heavier—so no major gene for large birth size is linked with agouti. When a second pair of allelomorphs, black and brown (B and b), are contrasted, the results are the same. The differences in favor of mice with the recessive b are $0.03 \pm .024$ and $0.01 \pm .025$ gram for males and females, respectively. The third pair of allelomorphs, intense and dilute (D and d), likewise show no significant difference (males, $0.01 \pm .025$; females, $0.06 \pm .025$ gram).

There is a possibility, however, that size at birth may be due to a number of cumulative genes linked to several qualitative genes each of which has had a slight effect. If such were the case, those animals possessing two or three recessive genes should be heavier than those with the two or three

dominant allelomorphs and slight linkage could be more easily detected. Table 3 gives no evidence for the existence of such a situation. In no case is the difference statistically significant. Thus, in this back-cross there is no indication that factors determining size at birth in mice are linked with any of the three qualitative characters involved. Kopeć ('24 a) found no evidence for the linkage of qualitative and quantitative characters in the newborn weight of rabbits.

Table 4 presents a summary of the data similarly arranged for the reciprocal back-cross. Such differences as are present are likewise slight and fluctuating and probably purely fortuitous, since in no case is a difference as great as three times its probable error. Thus again, there is no evidence for the linkage of genes for qualitative characters with the genes determining size at birth.

It will be observed that in every back-cross class, excepting the brown non-agoutis, males are heavier than females. In this one exception the means are identical.

CONCLUSIONS

The newborn weights of *Mus musculus* and *Mus bactrianus*, as revealed by averages, are specifically or racially characteristic.

The genetic complex of the growing fetus is more important than that of the mother in determining weight at birth.

There is no evidence that factors controlling weight at birth are linked with any of the three qualitative characters studied (dilution, brown, and non-agouti).

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